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Rocky Mountain Region

U.S. Department of Agriculture Forest Service

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ABSTRACT

Investigations were conducted to identify and characterize fungi associated with mortality of containerized Austrian pine seedlings within the Kansas State and Extension Forestry greenhouse in Manhattan. Necrosis began on the terminal buds and progressed down the main shoot resulting in seedling mortality. Major fungal associates included *Rhizoctonia* sp., *Cytospora* sp., *Verticillium* sp. and *Genicularia* sp. Pathogenicity tests required to determine etiology of the disease were not conducted. Possible ways to reduce future losses are discussed.

INTRODUCTION

The Kansas State and Extension Forestry greenhouse in Manhattan, Kansas is a relatively new facility designed to produce mostly containerized conifers for various planting projects throughout the State. Mortality of Austrian pine (*Pinus nigra* Arnold), a major coniferous species grown in the greenhouse, was noted in 1978 and *Rhizoctonia* sp. was commonly associated with diseased seedlings [1/] (Gary Boutz, personal communication). Other conifers commonly grown in the greenhouse include Scots pine (*Pinus sylvestris* L.) and eastern redcedar (*Juniperus virginiana* L.).

Mortality in a subsequent crop of seedlings grown during 1979 was investigated. Affected seedlings were examined in the greenhouse for symptoms and signs of disease and samples were collected for isolation of associated organisms. An attempt was also made to isolate potential pathogenic organisms from the growing medium used for containerized trees.

SYMPTOMATOLOGY

The first indication of disease was darkening of apical buds. Black sooty mold fungi often developed on affected buds. As the disease progressed, necrosis extended from the growing tips down through the shoots. Complete dieback with resultant seedling mortality was indicative of advanced stages of disease. Foliage drooped and infected tips often formed crooks (Fig. 1). Mortality of seedlings was usually rapid and occurred in groups. Fine, light gray mycelium was occasionally found on the aerial portions of recently killed trees.

MAJOR FUNGAL ASSOCIATES

Isolations were made from necrotic buds and terminal tissues with brown discoloration. Older, grey-colored needles were also

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1/ Diagnosis made by Extension Plant Pathology, Kansas State University, Manhattan.

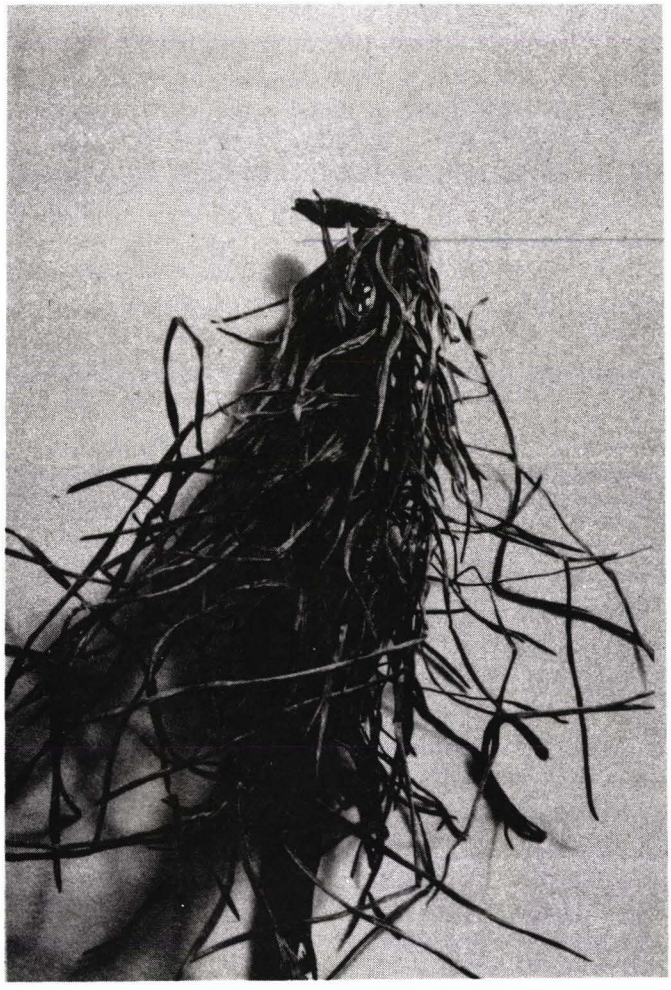

FIGURE 1. Necrotic Austrian pine seedling displaying needle droop and tip crook.

cultured for presence of fungi. Small amounts of growing medium were sprinkled over water agar for identification of resident fungi.

Rhizoctonia sp. was detected on necrotic terminal buds of affected seedlings. Sclerotia produced by the fungus (Fig. 2) measured about 40-70 µm in diameter and were embedded within bud scales. Sclerotia were oval and composed of loosely-packed, brown-pigmented hyphae wrapped around each other. Hyphae with septal constrictions and barrel-shaped cells (Fig. 3) typical of *Rhizoctonia* (2, 3) were found in close proximity to sclerotia.

Cytospora sp. was found on ashen-grey, necrotic needles. Highly stromatic, erumpent pycnidia were commonly produced when needles were incubated in a moist chamber for about 5 days. Pycnidia were black, ostiolate, and measured 100-180 µm in width by 180-250 µm in length. Conidia were one-celled, hyaline, elongate-curved (allantoid) and measured 4-6 µm in width by 14-20 µm in length. Conidia were exuded in a mass at the ostiolar tips of pycnidia.

Verticillium sp. was isolated from chlorotic and recently necrotic needles. Hyaline conidiophores were septate, penicillately-branched and produced apical groups of conidia in a slimy matrix. Conidia were hyaline, one-celled, oval to kidney-shaped, and measured 2-4 µm in width by 5-6 µm in length.

Hyaline conidiophores of *Genicularia* sp. were produced on necrotic terminal buds which were incubated in moist chambers for about 5 days. Conidiophores produced budding conidia, which were hyaline, two-celled, pear shaped and measured 4-6 µm in width by 15-20 µm in length.

Fungi isolated from growing media samples were restricted to the common soil mold genera *Trichoderma* and *Penicillium*.

DISCUSSION

Production of containerized seedlings within greenhouses often results in larger trees during a shorter period of time than traditional bare-root stock operations. However, such greenhouse operations may be plagued with disease problems (9). High relative humidity maintained for extended time periods may result in accumulation of free moisture on foliage. Seedlings also tend to be grown in very dense stands. This provides ideal conditions for infection and proliferation of foliar pathogens.

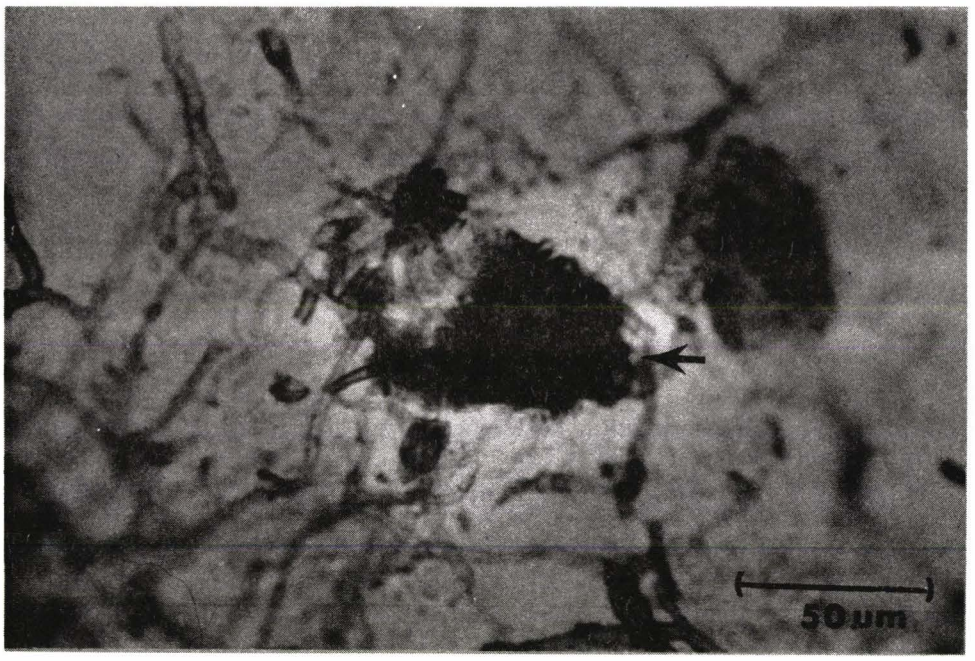

FIGURE 2. *Rhizoctonia* sclerotium (arrow) embedded within necrotic Austrian pine bud scale.

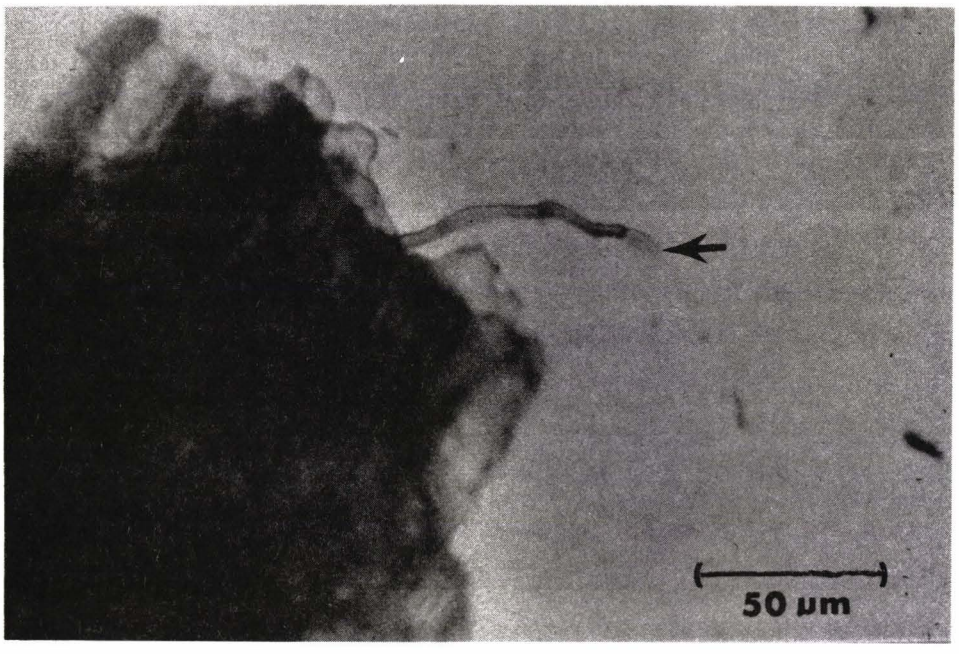

FIGURE 3. Barrel-shaped cell (arrow) on the terminus of a *Rhizoctonia* hypha with constricted septations.

Tip necrosis and morality of Austrian pine seedlings at the Kansas State and Extension Forestry greenhouse increased in severity shortly after ambient temperatures were reduced to 1^{o} C - 4^{o} C to provide cold hardiness to trees prior to their removal to the lathhouse. Lower temperatures may have reduced tree vigor or injured tissues so that seedlings were susceptible to facultative pathogens which would not normally cause disease. Symptoms produced on individual seedlings resembled those caused by *Sirococcus strobilinus* Preuss (8, 11) and group mortality was similar to that incited by *Botrytis cinerea* (Fr.) Pers. (5). However, neither of these suspected fungi were isolated from symptomatic seedlings.

Rhizoctonia sp. may play a role in mortality of these containerized Austrian pine seedlings. This genus comprises universal soil inhabitants with saprophytic, pathogenic and mycorrhizal forms (1). Some strains cause disease in the aerial portions of plants during warm, humid conditions. They may cause leaf spots, fruit and top rots, and bud killing. Baker (1) reported *R. solani* Kuhn causing bud necrosis and top rot of closely-spaced red pine (*Pinus resinosa* Ait.) seedlings. Infection apparently resulted from splashing soil infested with the pathogen during irrigation. Latham (6) found *Rhizoctonia* sp. causing considerable top killing of 1-2 year old red spruce seedlings in a West Virginia nursery. He described angular bending of stem tips as a conspicuous symptom. The disease was most severe during moist seasons in dense stands in which seedling tops were in close contact. Diseased Austrian pine seedlings displayed similar angular bending and mortality was greatest in dense stands.

Cytospora sp., which was also associated with Austrian pine mortality, has both parasitic and saprophytic forms (4). Parasitic strains primarily form cankers on both conifers and hardwoods (13, 15). Most members of this genus are considered inhabitants of bark tissues and may become parasitic during host stress (10). Since *Cytospora* sp. was commonly found only in moribund needle tissue and not from recently necrotic stems, its role in Austrian pine mortality is not known.

Verticillium spp. are mostly parasitic fungi which induce vascular wilts of dicotyledonous plants (14). The fungus may also be the imperfect state of *Nectria* which would implicate it in canker diseases (12). However, *Verticillium* sp. was not isolated from the vascular region or stem of Austrian pine seedlings, but rather was found as an epiphyte on chlorotic and recently necrotic needles. Therefore, the role of *Verticillium* sp. in seedling mortality is questionable.

Genicularia spp. are generally saprophytic on wood or parasitic on nematodes (2). It is doubtful if this fungus could incite the types of symptoms found on Austrian pine seedlings. It was most likely saprophytic on necrotic tissues.

Without properly conducted pathogenicity tests, the etiology of Austrian pine seedling tip necrosis and mortality cannot be positively resolved. However, association of *Rhizoctonia* sp. with symptomatic seedlings and history of its similar behavior on other conifers, strongly implicates this fungus as the causal agent.

Since isolations from seedling soil media failed to indicate presence of *Rhizoctonia* sp., the question of inoculum sources remains unanswered. The fungus produces sclerotia which retain viability for long periods in dead and decaying plant debris (3). Such debris on unsterilized benches may provide initial inoculum.

Disease-inciting roles of the other isolated fungi are probably secondary. There may be some synergistic disease interactions, particularly between *Rhizoctonia* sp. and *Verticillium* sp., since both fungi are highly pathogenic. Without further investigations, interactions among organisms in the pathology of Austrian pine seedlings remains speculative.

RECOMMENDATIONS

The following recommedations are offered to reduce future losses to Austrian pine containerized seedlings:

(1) All infected seedlings should be removed from the greenhouse as soon as they are discovered. Frequent inspections for symptoms are necessary in order to remove infected trees before they can serve as inoculum sources. Samples of diseased trees should be furnished to a diagnostic laboratory for etiology determination.

(2) All dead plant debris in and around containerized conifers should be removed outside the greenhouse environment.

(3) All benches should be surface sterilized with sodium hypochlocite (10%) or some other appropriate sterilant prior to use and after each tree crop is removed. It is important to maintain a "sanitary" greenhouse in order to reduce potential inoculum sources.

(4) Protective fungicidal application may be warranted depending upon level of infection found during symptom inspections. Pentachloronitrobenzene (PCNB) has a history of being effective against *Rhizoctonia* spp. (7). This, of course, assumes that *Rhizoctonia* sp. was the major cause of the disease. Further investigations, including pathogenicity tests are required to verify this conclusion. All pesticide label instructions should be followed during application.

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PESTICIDE USE STATEMENT

Pesticides used improperly can be injurious to man, animals, and plants. Follow the directions and heed all precautions on the labels.

Store pesticides in original containers under lock and key -- out of the reach of children and animals -- and away from food and feed.

Apply pesticides so that they do not endanger humans, livestock, crops, beneficial insects, fish, and wildlife. Do not apply pesticides when there is danger of drift, when honey bees or other pollinating insects are visiting plants, or in ways that may contaminate water or leave illegal residues.

Avoid prolonged inhalation of pesticide sprays or dusts; wear protective clothing and equipment if specified on the container.

If your hands become contaminated with a pesticide, do not eat or drink until you have washed. In case a pesticide is swallowed or gets in the eyes, follow the first aid treatment given on the label, and get prompt medical attention. If a pesticide is spilled on your skin or clothing, remove clothing immediately and wash skin thoroughly.

Do not clean spray equipment or dump excess spray material near ponds, streams, or wells. Because it is difficult to remove all traces of herbicides from equipment, do not use the same equipment for insecticides or fungicides that you use for herbicides.

Dispose of empty pesticide containers promptly. Have them buried at a sanitary land-fill dump, or crush and bury them in a level, isolated place.

NOTE: Some States have restrictions on the use of certain pesticides. Check your State and local regulations. Also, because registrations of pesticides are under constant review by the U. S. Department of Agriculture, consult your county agricultural agent or State Extension specialist to be sure the intended use is still registered.